

Supplementary Information

Aluminium fluorescence detection with a FRET amplified chemosensor

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(Total 8 pages including this cover page)

Table of Contents

1. Experimental Procedures	2
2. Synthesis of compounds 1 , 3 and 4	3
3. UV-Visible titration of ligand 3 with Al ³⁺ (Figure S1).....	6
4. UV-Visible titration of compound 1 with Al ³⁺ (Figure S2).....	6
5. UV-Visible spectra of ligand 3 at increasing pH values (Figure S3 and S4).....	7
6. Fluorescence emission spectra of sensor 1 and coumarin amide 4 (Figure S5)	8
7. Fluorescence response of compounds 1 to Al ³⁺ in the presence of other metal ions (Figure S6)	7

1. Experimental Procedures.

General: ^1H NMR spectra were run on a Jeol EX-400 instrument. Chemical shifts are reported relative to internal Me_4Si . Multiplicity is given as usual. ESI-MS spectra were obtained on a PE-API spectrometer at 5600 volts by infusion of methanolic solutions. UV-Vis absorption measurements were performed on a Perkin Elmer Lambda 16 spectrophotometer equipped with a thermostated cell holder. Fluorescence spectra were recorded on a Perkin Elmer LS-50B spectrometer equipped with a thermostated cell holder. TLC's were performed on Polygram[®] Sil G/UV₂₅₄ silica gel pre-coated plastic sheets. Flash chromatography was run on silica gel, 230-400 mesh ASTM (Kieselgel 60, Merck). Solvents were purified under standard techniques. Reagents were purchased by Aldrich and used as received. $\text{Al}(\text{NO}_3)_3$, $\text{Cu}(\text{NO}_3)_2$, $\text{Zn}(\text{NO}_3)_2$, $\text{Ni}(\text{NO}_3)_2$, FeCl_3 , CaCl_2 , MgCl_2 were analytical grade products. Metal ion stock solutions were titrated against EDTA following standard procedures. 4-[3,5-bis-(2-hydroxyphenyl)-1,2,4-triazol-1-yl]-benzoic acid¹ (**2**), N-BOC-1,2-diaminoethane² (**5**) were prepared by known procedures.

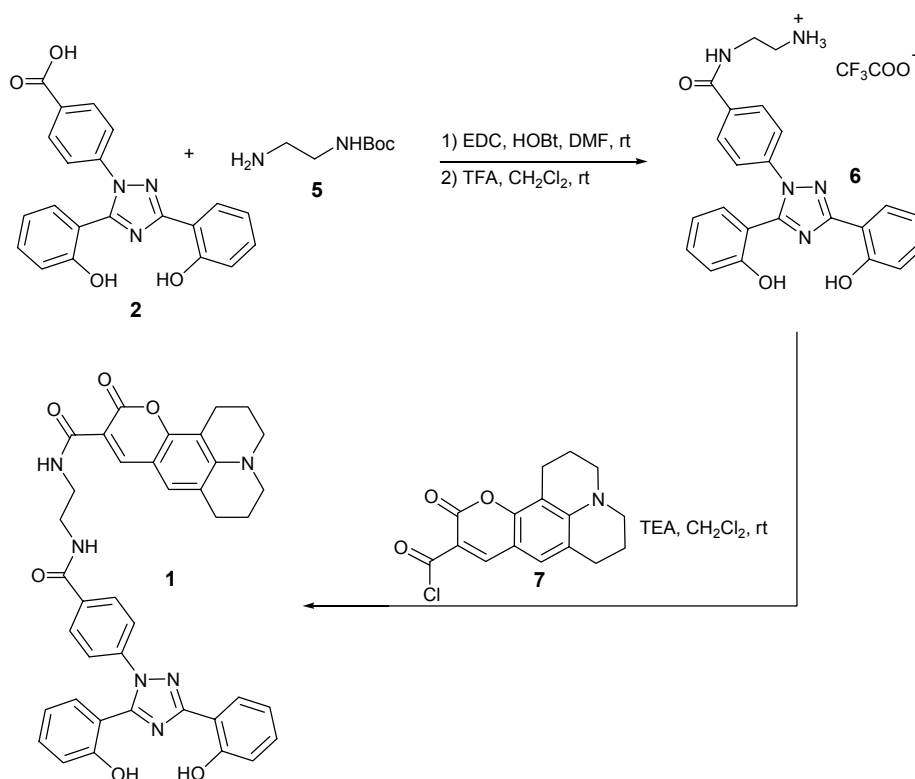
Spectrometric titrations: Several 2-mL solutions of buffer ($1 \cdot 10^{-2}$ M), ligands **1** or **3** ($3.11 \cdot 10^{-6}$ M in the case of fluorescence experiments or $0.9\text{-}1.6 \cdot 10^{-5}$ M in the case of the absorption experiments) and the desired amount of metal salt solution in water/ethanol 1:1 were prepared in plastic fluorescence cuvettes and incubated at 25° C for 12 hours. Complex formation was monitored following the absorption spectral changes. UV-Vis or fluorescence spectra were then recorded. From the spectral changes observed upon addition of the substrate, the K_{app} values were obtained by non-linear regression analyses of fluorescence data (at the selected wavelength) versus metal ion concentrations. The buffer used in the experiments was acetate buffer 0.01 M at pH 4.0. After mixing with ethanol the pH value read with the pH-meter was 5.0.

¹ R.Lattmann, P.Acklin (Novartis AG), WO-A 9749395A1 1997 [Chem.Abstr. 1998, **128**, 114953e]

² P.Krapcho, C.S.Kuell, *Synth. Commun.* 1990, **20**, 2559-2564.

2. Synthesis

2.1 Synthesis of derivative 1



A CH₂Cl₂ solution of HOBT (0.36g, 2.7 mmol) was added at r.t. to a solution of **2** (4-[3,5-bis-(2-hydroxyphenyl)-1,2,4-triazol-1-yl]-benzoic acid) (1g, 2.7 mmol) in 10 ml anhydrous DMF. After cooling down the solution to 0°C, EDC (0.062g, 3.2 mmol) was added, the mixture stirred for further 10 min at r.t., then a solution of **5** (mono t-butylloxycarbonyl-1,2-ethylenediamine) (0.43g, 2.7 mmol) and Et₃N (0.4ml, 3.2 mmol) in 10ml CH₂Cl₂ was added dropwise. The mixture was left on stirring, monitoring the course of the reaction by TLC (CHCl₃:EtOH 15:1). After 18h, the solution was diluted with CH₂Cl₂ (20ml), washed with 5% citric acid (3x) and water (5x). The combined organic layers were dried (Na₂SO₄), the solvent removed *in vacuo*, and the crude material was purified by FC (eluent: toluene-ethyl acetate 7:3) to give the product **6**, 0.260g, yield 19%.

¹H-NMR (400 MHz, MeOD) : δ 1.49 (s, 9H), 3.34 (t, 2H), 3.52 (t, 2H), 6.91 (d, 1H, J=8.0 Hz), 7.06 (m, 3H), 7.44 (m, 2H), 7.56 (dd, 1H, J = 7.7, 1.5 Hz), 7.62, 7.95 (AB quartet, 4H), 8.19 (dd, 1H, J 7.7, 1.5 Hz). ESI-MS calcd for C₂₈H₂₉N₅O₅ (M⁺): 515; obsd: 538 (M⁺ + Na⁺).

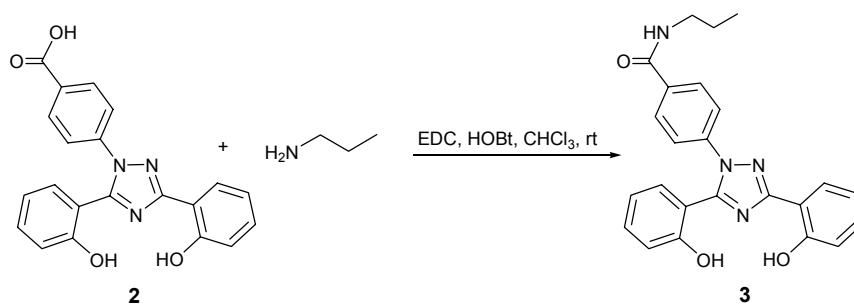
A solution of above Boc-protected derivative, (0.260g, 0.5 mmol) and TFA (10 ml) in CH₂Cl₂ (10ml) was stirred 1h at r.t. Evaporation of the solvent to dryness left a residue (product **6**) which was used without further purification. ¹H-NMR (400 MHz, MeOD), δ : 3.25 (t, 2H), 3.75 (t, 2H), 6.93 (d, 1H J = 8.4 Hz), 7.07 (m, 3H), 7.44 (m, 2H), 7.54 (dd, 1H J=7.3,1.5 Hz), 7.65, 8.00 (AB

quartet, 4H), 8.18 (dd, 1H, $J=7.7, 1.5$ Hz). ESI-MS calcd for $C_{23}H_{21}N_5O_3$ (M^+): 415; obsd: 438 ($M^+ + Na^+$).

Oxalylchloride (0.1 ml) and DMF (10 μ l) were added under Ar to a CH_2Cl_2 solution (10 ml) of Coumarin 343 (0.050 g 0.17 mmol). The solution was stirred at r.t. 1h, then the solvent was removed *in vacuo*. The resulting acid chloride **7**³ (0.050g, 0.16 mmol), dissolved in 10 ml CH_2Cl_2 , was dropwise added to a solution of **6** (0.086 g, 0.16 mmol) and Et_3N (0.075 ml, 0.52 mmol) in the same solvent. After stirring at room temperature 18h, the solution was extracted with H_2O , the organic phase dried and the solvent evaporated *in vacuo*. Purification by flash chromatography ($CH_2Cl_2/MeOH$ 10:1) gave the product **1**, 0.075 g, 67% yield.

¹H-NMR (400 MHz, $CDCl_3$): δ 1.97 (m, 4H), 2.76 (t, 2H), 2.89 (t, 2H), 3.34 (m, 4H), 3.71 (bt, 2H), 3.76 (bt, 2H), 6.65 (t, 1H), 6.94 (dd, 1H $J = 8.0, 1.5$ Hz), 7.00 (s, 1H), 7.06 (d, $J = 8.0$ Hz, 1H), 7.08 (d, $J = 8.04$ Hz, 1H), 7.14 (d, $J = 7.7$ Hz, 1H), 7.33 (m, 1H), 7.38 (m, 1H), 7.60, 8.10 (AB quartet, 4H), 8.14 (dd, 1H, $J=8.0, 1.8$ Hz), 8.38 (bt, 1H), 8.61 (s, 1H), 9.38 (t, 1H), 9.64 (s, 1H), 11.44 (s, 1H). ESI-MS calcd for $C_{39}H_{34}N_6O_6$ (M^+): 682; obsd: 705 ($M^+ + Na^+$).

2.2 Synthesis of ligand derivative 3

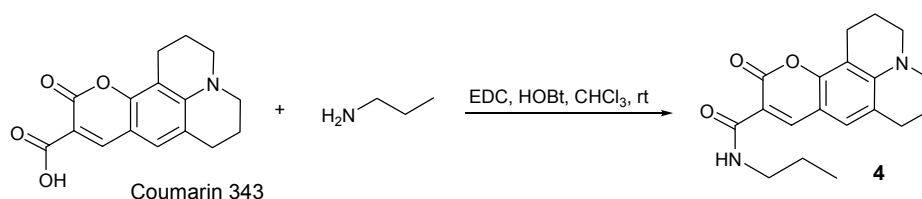


2 (60 mg, 0.16 mmol) was suspended in 4 ml of $CHCl_3$, triethylamine (0.10 mL, 0.72 mmol) and hydroxybenzotriazole (58 mg, 0.43 mmol) were added and the reaction mixture was cooled to 0° C. Then EDC·HCl (80 mg, 0.42 mmol) was added and, after 10 minutes, *n*-propylamine (0.020 mL, 0.47 mmol). The reaction mixture was stirred at 0° C for 1 hour and at room temperature overnight. After this time, 100 mL of CH_2Cl_2 were added and the mixture was extracted with a 5% $KHSO_4$ solution (2x), a 5% $NaHCO_3$ solution (2x) and water (2x). The organic phase was dried ($NaSO_4$) and the solvent removed to yield 62 mg (94%) of **3** as a white solid.

¹H NMR ($CDCl_3$) δ : 1.03 (t, $J = 7.2$ Hz, 3H), 1.68 (m, 2 H), 3.47 (t, $J = 6.3$ Hz, 2H), 6.2 (bs, 1H), 6.6 (t, $J = 7.0$ Hz, 1H), 7.4-6.9 (m, 6H), 7.60 (d, $J = 8.1$ Hz, 2H), 7.96 (d, $J = 8.1$ Hz, 2H), 8.16 (d, $J = 7.0$ Hz, 1H). ESI-MS calcd for $C_{24}H_{22}N_4O_3$ (M^+): 414; obsd: 437 ($M^+ + Na^+$).

³ J Gompel, G.Schuster, *J.Org.Chem.* 1987, **52**, 1465-1468.

2.2 Synthesis of coumarine derivative 4



Coumarin 343 (30 mg, 0.11 mmol), triethylamine (0.70 mL, 0.49 mmol) and hydroxybenzotriazole (43 mg, 0.32 mmol) were dissolved in 2 ml of CHCl₃ and the reaction mixture was cooled to 0° C. Then EDC·HCl (64 mg, 0.32 mmol) was added and, after 10 minutes, *n*-propylamine (0.014 mL, 0.32 mmol). The reaction mixture was stirred at 0° C for 1 hour and at room temperature overnight. After this time, 100 mL of CH₂Cl₂ were added and the mixture was extracted with a 5% KHSO₄ solution (2x), a 5% NaHCO₃ solution (2x) and water (2x). The organic phase was dried (Na₂SO₄) and the solvent removed. The crude product was purified by flash column chromatography (silica gel, CH₂Cl₂/CH₃OH 20:1) to yield 35 mg (97%) of **4** as a white solid.

¹H NMR (CDCl₃) δ: 0.97 (t, *J* = 7.5 Hz, 3H), 1.62 (m, 2 H), 1.96 (m, 4H), 2.76 (t, *J* = 6.3 Hz, 2H), 2.87 (t, *J* = 6.5 Hz, 2H), 3.3-3.4 (m, 6H), 7.03 (s, 1H), 8.64 (s, 1H), 9.04 (bs, 1H). ESI-MS calcd for C₁₉H₂₂N₂O₃ (M⁺): 326; obsd: 349 (M⁺ + Na⁺).

3. UV-Visible titration of ligand **3** with Al^{3+}

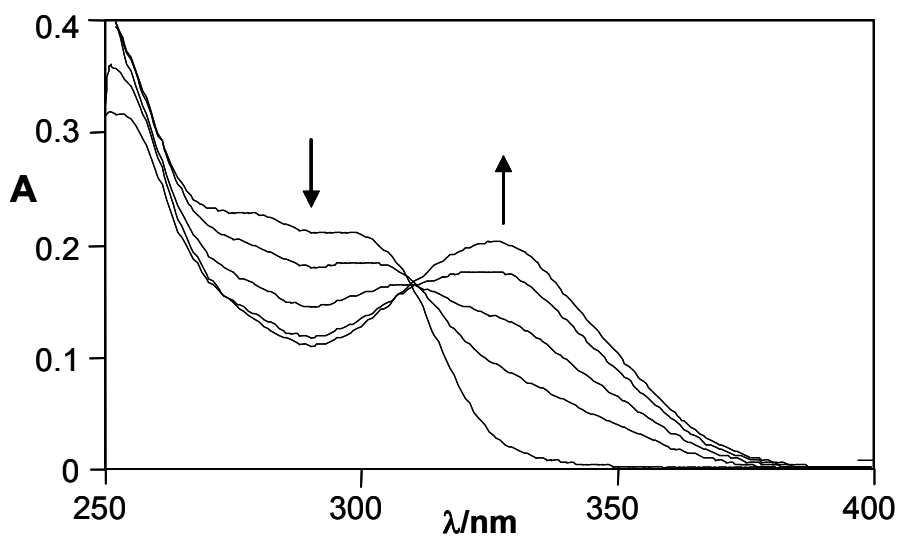


Figure S1: UV-Visible spectra of ligand **3** in the presence of increasing amounts of $\text{Al}(\text{NO}_3)_3$ in EtOH/ H_2O (1/1) at pH= 5.0. [**3**]= 8.7×10^{-6} M, [acetate buffer] = 0.01M.

4. UV-Visible titration of compound **1** with Al^{3+}

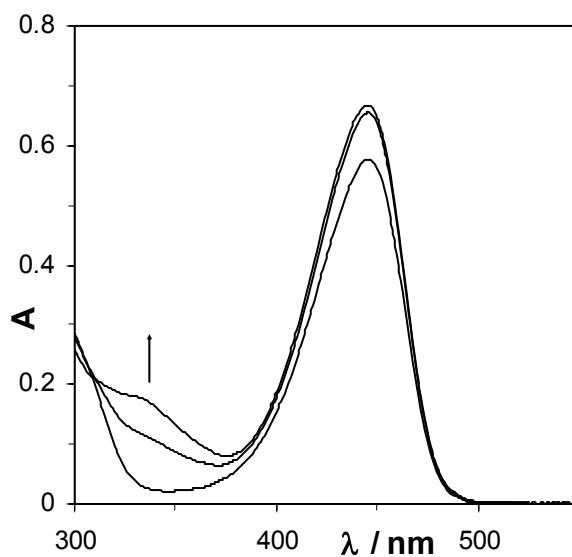


Figure S2: UV-Visible spectra of compound **1** in the presence of increasing amounts of $\text{Al}(\text{NO}_3)_3$ in EtOH/ H_2O (1/1) at pH= 5.0. [**1**]= 1.65×10^{-5} M, [acetate buffer] = 0.01M.

5. UV-Visible spectra of ligand **3** at increasing pH values

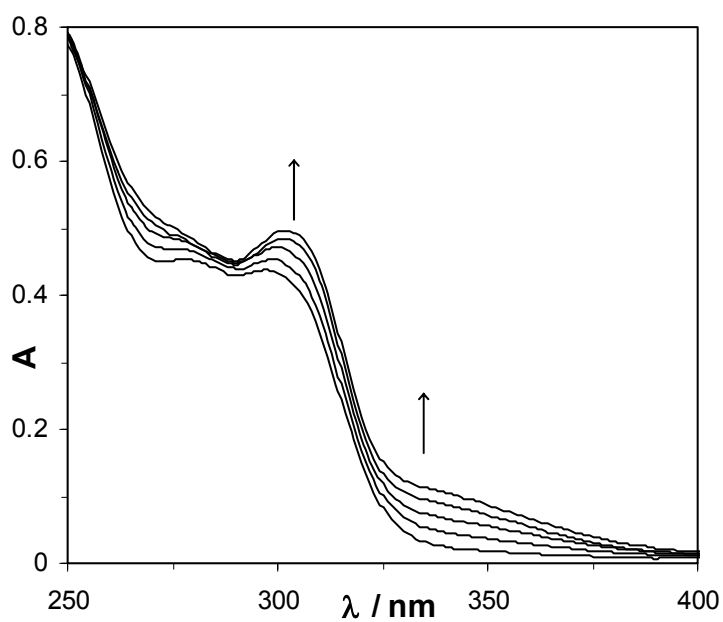


Figure S3: UV-Visible spectra of ligand **3** ($1.74 \cdot 10^{-5}$ M) at increasing pH values in the range 7-12 in EtOH/H₂O 1:1.

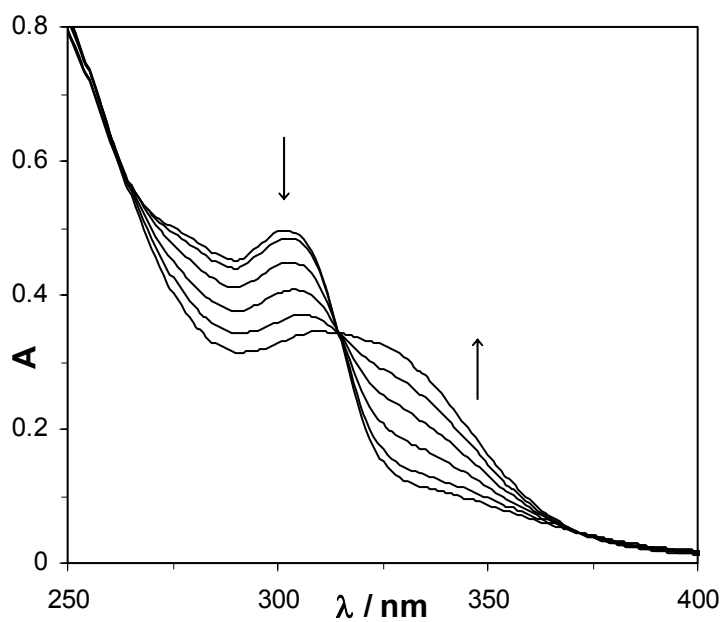


Figure S4: UV-Visible spectra of ligand **3** ($1.74 \cdot 10^{-5}$ M) at increasing pH values in the range 12-13.9 in EtOH/H₂O 1:1.

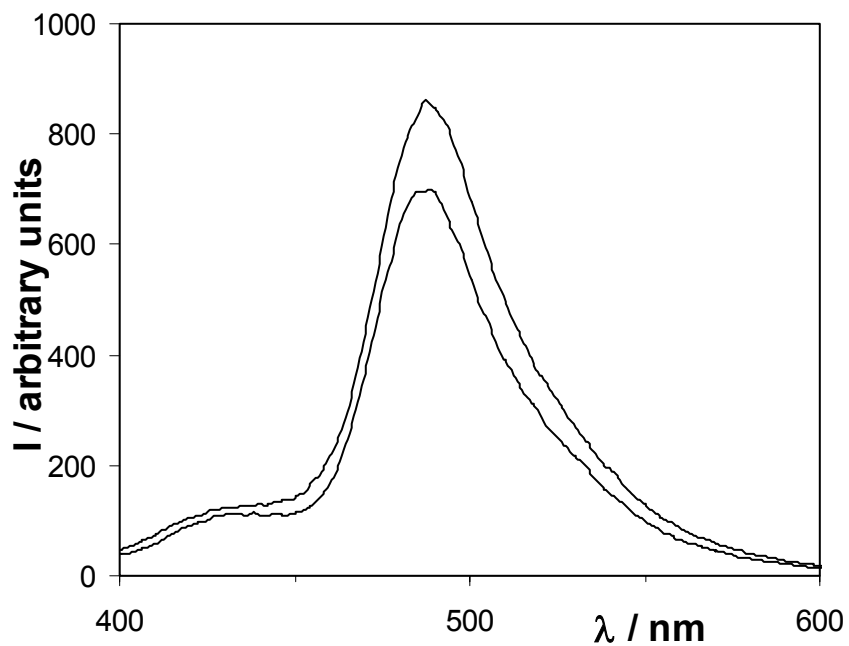


Figure S5: Fluorescence emission spectra ($\lambda_{\text{exc}} = 445 \text{ nm}$) of sensor **1** (upper curve) and coumarin derivative **4** (lower curve) in ethanol. $[\mathbf{1}] = [\mathbf{4}] = 3.11 \cdot 10^{-6} \text{ M}$.

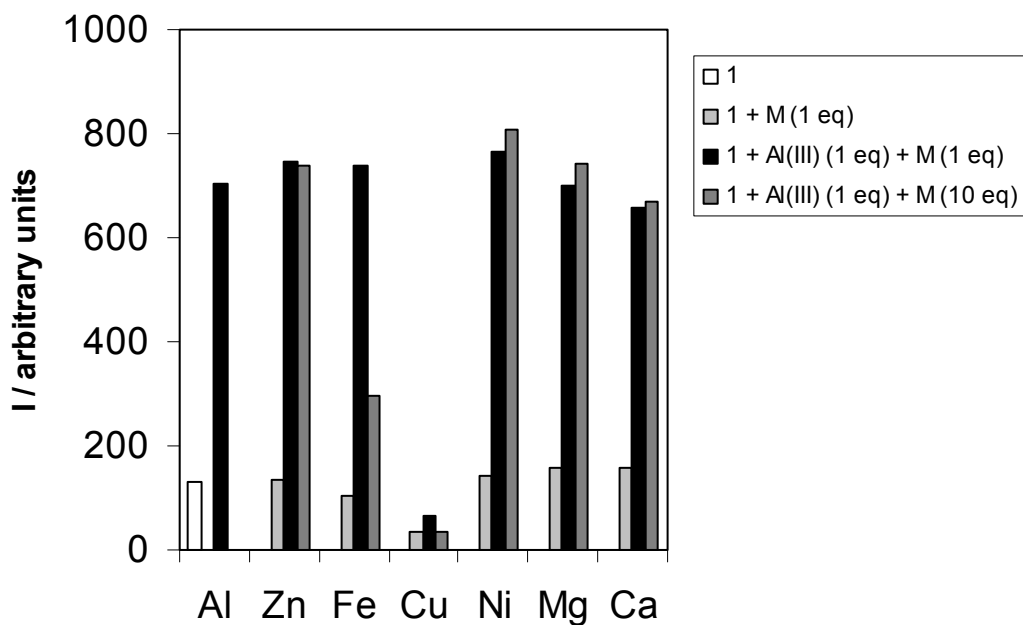


Figure S6: Fluorescence response of chemosensor **1** to Al^{3+} in the presence of other metal ions. Conditions: EtOH/H₂O (1/1), pH= 5.0. $[\mathbf{1}] = 3.0 \times 10^{-6} \text{ M}$, [acetate buffer] = 0.01M.